



PCT/US00/22743

-54-

CLAIMS

What is claimed is:

1. A method of targeted cloning and enrichment of genes and gene clusters by:

directly isolating and subsequent cloning the targeted genes/cluster.

2. The method according to claim 1, wherein said isolating step includes the steps of:

creating a primer containing a target oligonucleotide; adding the primer to a sample of DNA; and performing PCR to replicate the genes targeted by the primer.

3. The method according to claim 2, wherein said creating step further

includes creating a primer using k-tuple, template derivation and

- degenerate PCR.
- 4. The method according to claim 2, wherein said performing step further includes performing degenerate, nested, and temperature gradient PCR.
- 5. A primer for use in the method of claim1.
- 6. The primer according to claim 5, wherein said primer is selected from Table I.
- 7. A method of isolating trimethoprim coding genes by directly isolating and subsequent cloning the trimethoprim coding gene.
- 8. The method according to claim 7, wherein said isolating step includes the steps of:

creating a primer containing a target oligonucleotide using a method selected from the group consisting of k-tuple, template derivation and degenerate PCR;



WO 01/12861





PCT/US00/22743

- adding the primer to a sample of DNA; and performing PCR to replicate the trimethoprim coding gene targeted by the primer.
- The method according in claim 8, wherein said creating step further includes creating a primer containing a target oligonucleotide coding for DHFR2.
- 10. Probes for use in the method according to claim 1.
- 11. The probes according to claim 10, wherein said probes are selected from Table II.
- 12. Genes cloned by the method according to claim.
- 13. The genes according to claim 12, wherein said genes are selected from Table II.
- 14. A library formed by the method of claim 1.
- 15. A method of providing degenerate cloning of an entire family of genes from a mixed DNA sample by directly isolating and subsequent cloning targeted genes/clusters.
- 16. The method according to claim 15, wherein said isolating step includes the steps of:

degenerately cloning a target oligonucleotide, creating a primer containing the target oligonucleotide; adding the primer to a mixed sample of DNA; and performing PCR to replicate the genes targeted by the primer.

- 17. Genes cloned according to the method of claim 15 for use in affinity purification of genes.
- 18. The genes according to claim 17, further including using said genes for cloning associated biosynthetic pathway genes.

Substitute Sheet (Rule 26)